Background

In this guide

In this guide

Definitions

12. The terms ‘herbal infusions’ and ‘tea (*Camellia sinensis*; *C. sinensis*)’ as defined in Annex of Commission Regulation (EU) 2020/2040 refer to: herbal infusions (dried product) from flowers, leaves, herbs, roots and any other parts of the plant (in sachets or in bulk)/tea (*C. sinensis*) (dried product) from dried leaves, stalks and flowers (in sachets or in bulk) used for the preparation of herbal infusion (liquid product)/tea (liquid product) (UK Legislation, 2020).

13. The term ‘green tea extract’ refers to ‘herbal infusions’ which have been soaked in ethanol/water mixtures after which they have been concentrated to 40-50% solids (as powders or dry extracts) (UK Legislation, 2020).

Identity

14. Green tea, produced from the leaves of the *C. sinensis* plant, is a popular drink, consumed worldwide. Various reports on the health benefits of

green tea on different types of cancer, liver and heart disease are available in the literature. Many of these claimed beneficial effects are associated with a particular catechin, epigallocatechin-3-gallate (EGCG) (Chacko et al., 2010).

15. Catechins are polyphenolic compounds, and most of the polyphenols derived from green tea are catechins. GTCs are derived from the unfermented leaves and leaf buds of the tea plant, *C. sinensis*. Catechins constitute ~20% of the total flavonoids found in green tea (Sakata et al., 2013). Aqueous alcohol extraction of *C. sinensis* leaves concentrates levels of catechins and removes other components such as caffeine (*C. sinensis* has naturally occurring caffeine; Vuong & Roach, 2014).

16. As identified by EFSA, the most significant catechin associated with hepatotoxicity is epigallocatechin-3-gallate (EGCG) (EFSA, 2018).

17. A study by Bhagwat et al., reported that EGCG is present in dried leaves of green tea at around 7,400 mg per 100 g (limited sample number) (Bhagwat et al, 2011). Khan et al., 2006 have reported EGCG levels of 200-300 mg in a cup of brewed green tea. EGCG has been reported to form more than 50% of all GTC, representing ~16.5% of the water-extractable fraction of tea (Balentine et al., 1997). EGCG is present in green tea infusions but is found at a higher concentration in GTEs.

Toxic mode of action

18. The exact mechanism of GTE associated liver injury has not yet been fully elucidated. There is evidence that exposure to GTCs (specifically EGCG) can result in increased serum levels of the enzymes aspartate transaminase (AST) and alanine transaminase (ALT), which are biomarkers of liver toxicity (Gurley et al., 2022). For example, in a prospective study in post-menopausal women, raised ALT levels were found in 6.7% of treated subjects and in only 0.7% of the controls. Most instances of liver toxicity were mild but, only in the treated group, a few were serious (Acosta et al., 2022, further described in paragraph 117).

19. Shil et al., (2022) investigated the effects of two decaffeinated GTEs (dGTE) on oxidative stress, mitochondrial function and cell viability in HepG2 cells. dGTE1 was sourced from a food ingredient manufacturer and was an anhydrous capsulated powder standardised to 70% EGCG; each capsule provided a total 725 mg dGTE1, of which 400 mg was EGCG. dGTE2 was sourced directly from a supplement company and was also an anhydrous powder standardised to 98% polyphenols; each capsule provided 725 mg dGTE2, of which 326 mg was

EGCG. Cells were exposed to dGTE concentrations ranging from 0.001 to 1000 µg/ml. dGTE1 was found to be protective against hydrogen peroxide-induced apoptosis and cell death by attenuating oxidative stress pathways. In contrast to dGTE2, which increased cellular and mitochondrial oxidative stress and apoptosis itself, and if anything, exacerbated that induced by hydrogen peroxide. The effects of dGTE1 resembled those of EGCG itself. The differences between the two dGTE preparations were attributed to the presence of constituents other than EGCG in dGTE2.

20. Shi et al., (2021) aimed to characterise the metabolic alterations induced by EGCG and the effects of dietary restriction in female C57BL/6J mice plasma using metabolomics. Mice were split into two groups and were fed either a free or fixed diet (50% of the average food intake, limited to 2 g each mouse per day) once daily for six days. On the final day, the mice in the free diet group were further divided into three groups (n=8/group): control, 400 mg EGCG/kg [bw] and 800 mg EGCG/kg [bw]. The mice in the fixed diet group were also divided into three groups (n=8/group): fixed diet group control, fixed diet with 400 mg EGCG/kg [bw] and fixed diet with 800 mg EGCG/kg [bw]. Note that the EGCG (>98% purity) treatment groups were dosed via intragastric administration. Further note that the doses were assumed to have been expressed per kg body weight; however, the methodology did not clarify this in detail. The results showed that EGCG induced enhanced lipid metabolism pathways, although EGCG did not cause liver injury at the doses used in animals that were not on a restricted diet. In mice that were under dietary restrictions, it was observed that EGCG caused dose-dependent hepatotoxicity and was associated with the overactivation of linoleic acid and arachidonic acid oxidation pathways, which increased the accumulation of pro-inflammatory lipid metabolites and thus contribute to liver injury.

21. Pandey et al., (2020) investigated the potential inhibition of human nicotinamide adenine dinucleotide phosphate-quinone oxidoreductase 1 (NQO1), an enzyme that plays a role in oxidative stress, by EGCG and its metabolites using a systematic computational approach. This included molecular docking, binding free energy calculations, and molecular dynamics simulations. It has been reported that *o*-quinone metabolites of gallic acid or EGCG are causative agents for the observed hepatotoxicity in humans. The binding free energy calculations showed that some EGCG metabolites exhibited strong predicted binding affinity to NQO1. The authors concluded that this may explain the observed idiosyncratic hepatotoxicity caused by the consumption of green tea and its constituents. However, the authors appreciate that there is a need for experimental validation

of their results with appropriate biological methods. It is not clear any hepatotoxicity resulting from the interactions described would be idiosyncratic.

22. GTCs have been reported to modulate epigenetic processes, such as DNA methylation, which play a significant role in cancer development. Yiannakopoulu (2015) published a review on the modulation of DNA methylation by GTCs. It was found that EGCG modulates DNA methylation by attenuating the effect of DNA methyltransferase 1 (DNMT1). However, the exact mechanism of DNMT1 inhibition is not fully understood. The potential mechanisms include (in)direct enzymatic inhibition, reduced DNMT1 expression and translation. The inhibition of DNMT1 is expected to prevent hypermethylation (thus, seen as a therapeutic action); however, in cases of severe reduction of DNMT activity, it may cause DNA hypomethylation, genomic instability and early development of cancers such as T-cell lymphomas and sarcomas (Eden et al., 2003; Gaudet et al., 2003).

23. Sergi (2020) reviewed the toxicity of EGCG in children. In general, EGCG demonstrated poor oral absorption, even at a daily intake corresponding to 8-16 cups of green tea. EGCG blood levels peak within 1.7 hours after consumption and it has a plasma half-life of ~5 hours (in healthy adults) (Lee *et al*., (2002); Chow *et al*., (2003)). EGCG toxicity observed at high concentrations was related to pro-oxidative properties attributed to the catechol structures (ortho-diphenols). These structures are able to form a superoxide anion radical ($O2^{-}$) from molecular oxygen using an electrophilic *o*-quinone functional group. The ester group in EGCG may also interact with a cell's lipid bi-layer membrane. The effects of EGCG on the mitochondria were also described. EGCG promoted decreased reactive oxygen species (ROS) production on low doses, but increased ROS formation on high doses. EGCG seemingly caused damage to the outer mitochondrial membrane and uncoupling of oxidative phosphorylation in permeabilized hepatocytes. Sergi concluded that the use of green tea polyphenols (including EGCG) should not be considered for use in children because of the potential adverse effect on immature or fast-developing liver cells.

24. Bedrood et al., (2018) performed a review on the toxicological effects of *Camellia sinensis* (green tea). The most important side effects of green tea and its constituents (e.g., polyphenols) to have been reported were hepatotoxicity and gastrointestinal disorders especially when consumed on an empty stomach. Inhibitory effects on metabolising enzymes and intestinal absorption as well as nervous system stimulatory effects were also reported. Green tea and its main components were not major teratogenic, mutagenic or carcinogenic substances.

It was noted by the authors, that there are limited data on using green tea and its components during pregnancy, and that they should be used with caution during pregnancy, breast-feeding and in susceptible people. In addition, green tea and its components show a wide variety of drug-interactions, and consideration should be taken when co-administrating with drugs.

25. James et al., (2018) examined the hepatotoxic effects of EGCG in C57BL/6J mice and evaluated changes in hepatic antioxidant response and mitochondria structure and function. Mice (n=11-18 per group) were dosed with EGCG (93% pure at up to 750 mg/kg) once daily for 3 days via intragastric administration. Hepatic inflammation, necrosis and haemorrhage were observed. Hepatotoxicity was associated with increased oxidative stress and decreased superoxide dismutase and glutathione peroxidase levels. The authors concluded that their data indicate the mitochondria may be a target for EGCG, and that the inhibition of mitochondria function/antioxidant response may be important for the hepatotoxicity of bolus exposure to EGCG.

26. The above studies are summarised in Table 1 in Annex B.

27. EFSA noted that rare cases of liver injury have been reported after consumption of green tea infusions, most probably due to an idiosyncratic reaction. Overall, the EFSA Panel noted that the number of human cases with hepatotoxicity associated with green tea infusions is extremely low when compared to the large number of consumers of green tea infusions (EFSA, 2018).

Chemical characterisation

28. Chemical analysis shows that green tea leaves contain several other constituents in addition to catechins. Some of these constituents, such as caffeine, could alter the toxicological profile of catechins. The difference in constituents and/or concentration levels of catechins observed may be due to varying growth conditions, manufacturing processes, product shelf-life and storage conditions (Jafaar et al., 2017).

29. In other instances, for the broad term of GTE, the composition of this extract remains unknown. In 2020, an FSA survey detected the presence of pyrrolizidine alkaloids (PAs) in 11 out of 55 samples from *C. sinensis,* typically at levels of 500 µg per kg, most likely due to co-harvesting with PA producing plants (FSA, 2020).

30. PAs can result in hepatotoxicity following both acute and chronic exposure (COT, 2008; EFSA, 2017). The toxicity of PAs is almost exclusively associated with their metabolites forming DNA adducts. Pyrroles can penetrate the nucleus and react with DNA leading to the formation of DNA cross-links and DNA-protein cross-links. In the liver, they can pass to the adjacent space of Disse and into the sinusoidal lumen, where they interact with sinusoidal cells.

31. The adverse toxicological effects and potency of PAs can be considered an uncertainty when assessing the possible hepatotoxicity of GTCs (in either green tea infusions or GTEs in supplements), as they can lead to similar hepatotoxicity endpoints, and therefore uncertainty exists as to what degree of hepatotoxicity is caused by PAs as compared to GTCs. However, the COT noted that the primary effect of PAs in the liver is most often characterised by hepatic veno-occlusive disease. Hence, there are differences in the hepatopathology profile observed with PAs and GTCs. This would suggest that any contribution of PAs is more likely to be synergistic than as an alternative hepatotoxin. PAs are discussed in more detail in paragraphs 68 - 79.

32. Green tea has also been shown to contain residues of pesticides and contaminants such as mycotoxins and heavy metals (Abd El-Aty et al., 2014). These substances are usually subject to regulatory limits, or not detectable following leaching into an infusion or manufacture of GTEs (for use in supplements).

Previous evaluations

International Agency for Research on Cancer

33. The International Agency for Research on Cancer (IARC) concluded that there was inadequate evidence to conclude on the carcinogenicity in humans from tea drinking. There was also inadequate evidence to conclude on the carcinogenicity in experimental animals exposed to tea. Overall, IARC concluded that tea, which included green tea, is not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 1991).

United States Pharmacopeia

34. In 2007, the United States Pharmacopeia (USP) Dietary Supplement Information Expert Committee assigned a warning statement for GTE, based on reports of liver injury, which was later removed in 2009, following the review of

additional information and stakeholder comments (USP, 2009). More recently, the USP has included a cautionary labeling requirement in its powdered decaffeinated green tea extract monograph: “Do not take on an empty stomach. Take with food. Do not use if you have a liver problem and discontinue use and consult a healthcare practitioner if you develop symptoms of liver trouble, such as abdominal pain, dark urine, or jaundice (yellowing of the skin or eyes).” (Oketch-Rabah et al., 2020).

European Medicines Agency

35. The European Medicines Agency (EMA) identified contraindications for use of green tea herbal infusions, those being: hypersensitivity to the active substance(s), gastric and duodenal ulcers, cardiovascular disorders such as hypertension and arrhythmia and hyperthyroidism. Overdose was considered in the context of caffeine content and can lead to restlessness, tremor, and elevated excitability (quantities corresponding to more than 300 mg of caffeine or 5 cups of tea as a beverage). Green tea dried extracts were involved in some cases of hepatoxicity and gave rise to safety concerns. Hepatotoxicity is related to high doses of herbal preparations (up to 35% of catechins) and the cytotoxicity of EGCG exhibited at these levels (EMA, 2013).

European Food Safety Authority

36. An evaluation was carried out by EFSA (EFSA, 2018) on the safety of GTCs. The evaluation focused on GTCs and the associated cases of probable idiosyncratic hepatotoxicity. It was not a general safety assessment of either GTCs or green tea infusions and extracts. The EFSA opinion is summarised in the next section.

37. In 2018, following a series of reports of adverse effects as a result of the intake of GTE supplements, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) considered the possible association between the consumption of EGCG (the major catechin present in green tea) and hepatotoxicity (EFSA, 2018). Information on the metabolism of tea flavanols addressed in the EFSA opinion is based on data presented by the Norwegian Institute of Public Health (NIPH) (NIPH, 2015).

38. When comparing studies, the EFSA Panel considered supplements in terms of EGCG content, the principle catechin in green tea. EGCG has been shown to be more cytotoxic than both epigallocatechin (EGC) and epicatechin-3-gallate (ECG) in primary rat hepatocytes (Schmidt et al., 2005; Galati et al., 2006). It was

noted that in some cases, EGCG was the only catechin for which content was reported.

39. The EFSA Panel observed that generally, catechin metabolism follows the same pathway in mice, rats and humans based on the similarities in circulating, hepatic and intestinal metabolites observed in the plasma. Overall, animal models are generally predictive of catechin toxicokinetics in humans; however, due to other factors that affect toxicokinetics, there may be different outcomes. For example, GTCs are known to bind to dietary components such as proteins leading to a possible decrease in bioavailability of both catechins and dietary components, such as proteins and thus decreasing the free concentration available for absorption. Fasting was demonstrated to result in increased toxicity, probably due, in part, to a higher bioavailability of GTCs and reduced hepatic glycogen levels (which may be due to less binding of catechins to dietary proteins). Animal experiments suggest the liver is the primary target organ for toxicity.

40. The EFSA Panel noted that there were no specifications for the preparation of green tea as a food or food supplements in the EU Regulations and no monographs were held in the current edition of the European Pharmacopoeia. The Panel also noted the absence of a maximum limit for pyrrolizidine alkaloids in green tea preparation in food supplements.

41. However, the EFSA Opinion did note that the 2017 United States pharmacopoeia, provided specifications for 'Powdered decaffeinated Green Tea Extract' for use in green tea supplements, with the following definition: Powdered decaffeinated GTE is prepared from young unfermented leaves and the leaf buds of C. sinensis (L.) Kuntze (Family Theaceae) [syn, Thea sinesis L.] using suitable solvents such as alcohol, methanol, acetone, water, or mixtures of these solvents; the caffeine has been removed. The ratio of the starting crude plant material to powdered decaffeinated GTE is 6:1 to 10:1. It contains no less than 60% of polyphenols, calculated as (−)-epigallocatechin-3-O-gallate, no less than 40.0% of (−)-epigallocatechin-3-O-gallate, and no more than 0.1% of caffeine, all calculated on the anhydrous basis (USP-NF, 2023).

Toxicological data

42. As part of their assessment, EFSA reviewed literature studies, monographs and risk assessment reports available up to January 2018 following a public call for data (no data were received from interested parties on the levels of catechins in GTEs used for the manufacturing of food supplements). The risk

assessment was carried out according to the EFSA Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA, 2009). The Panel estimated chronic exposure to EGCG for the following population groups: infants, toddlers, children, adolescents, adults and the elderly.

43. In a peer reviewed publication based on human and animal data, a tolerable upper intake level (TUL) of 300 mg per day of EGCG was proposed by Yates et al., (2017) based on separate data from animals and in healthy human adults, respectively. An acceptable daily intake (ADI) of 4.6 mg/kg bw per day, derived from toxicity data, was proposed.

44. In a safety assessment of GTE supplements, Dekant et al., (2017) proposed a TUL of EGCG of 300 mg per person, based on clinical trials not reporting any liver effects (using a two-fold safety margin), and No Observed Adverse Effect Levels (NOAELs) from animal studies of dietary administration of GTCs (using a safety factor of 100).

45. There was evidence that intake of doses above 800 mg of EGCG per day over a duration of 4 months or longer led to elevations in ALT and AST levels in less than 10% of the general population (EFSA, 2018). Intense exercise can also increase serum ALT levels, and GTE supplements are often used in conjunction with other supplements by gym users, with the combined effect being unknown. In a smaller section of the general population (5.1%), doses of 843 mg EGCG per day over the course of a year, resulted in more serious effects on liver function. Additional factors contributing to hepatotoxicity were investigated, such as alcohol consumption, concomitant use of medicines and catechol-*O*-methyltransferase (COMT) genotype but these were not found to influence the hepatotoxicity. This effect was particularly noted in individuals with a higher body mass index, who were more likely to take weight loss supplements containing GTEs (Dostal et al., 2015; Yu et al., 2017).